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PRINCIPAL INVESTIGATOR: Nicanor Obaldia III, D.V.M.

CONTRACTING ORGANIZATION: PROMED Trading, S.A.
Miami, Florida 33126

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13. ABSTRACT *(Maximum 200 words)*

The *Plasmodium falciparum* 1088 strain did not adapt in *Aotus lemurinus lemurinus* when inoculated intraperitoneally. A Salvador I strain of *Plasmodium vivax* was adapted to splenectomized and intact Aotus after serial passage. Artelinic acid WR255663AK (JN8331) when given to Aotus by the oral route at 20 mg/kg b.i.d. for three days, appeared to be safe, and cleared a *P. falciparum* FVO infection. Re-treatment at 40 mg/kg cured a recrudecence that occurred 31 days PI. In vaccine studies, neither, Aotus immunized intradermally (ID) with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines alone or in combination, nor, Aotus immunized ID with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines as a combination with or without aGM-CSF, were protected against a challenge with an FVO strain of *P. falciparum.* However, when AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines were given ID as a combination to *P. falciparum* FVO cured Aotus, 4/6 (67%) animals were protected against an homologous infection. Total absence of antibody responses were observed in Aotus after three ID immunizations with *P. vivax* DNA vaccines based on PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP(regionsII-IV) alone or in combination. Homologous re-challenge with Vietnam-Oak Knoll parasites resulted in thirteen Aotus with sterile immunity after 4-6 inoculations.

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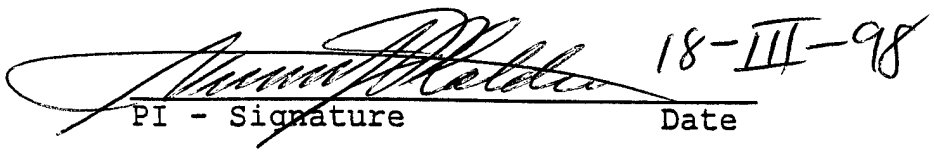

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INTRODUCTION

Each year there are 300-500 million new infections and 2-5 million deaths attributable to malaria that occur primarily in countries in the tropics, particularly in sub-Saharan Africa **(4).** During the past 10-20 years the malaria problem has intensified in some parts of the world because parasites have developed resistance to drugs used for treatment and prevention; the anopheles mosquito, which transmits the parasite to humans, has developed resistance to insecticides, and control efforts have been reduced as resources have diminished in some developing countries **(5).**

The use of *Aotus lemurinus lemurinus* (Panamanian Aotus monkey), cariotypes VIII and IX (11) as a model to study malaria drug resistance and vaccine efficacy, have been ongoing at Gorgas Memorial Laboratory since 1976, due in part to the availability of this monkey in Panama **(15)**, and also to the increasing drug resistance exhibited by the highly pathogenic *Plasmodium falciparum* parasites in Asia, Africa, and Latin America, and more recently *Plasmodium vivax* in the Melanesian and Indonesian archipelago **(16)**. Previously, Schmidt **(21, 22)** used the Colombian *Aotus* as the experimental host for antimalarial drug studies, but embargoes imposed by South American countries on the exportation of monkeys in the mid 1970's seriously restricted the use of *Aotus* for biomedical research in the United States, and in 1976 the project was transferred to Gorgas Memorial Laboratory where Panamanian *Aotus* were available for research. Since then, three strains of *P. falciparum*, Vietnam Smith, Uganda Palo Alto, and Vietnam Oak Knoll, had been adapted to Panamanian *Aotus*. These strains exhibit diverse susceptibility and/or resistance to standard antimalarial agents.

The course of untreated infections in Panamanian *Aotus* has been characterized and compared with that in *Aotus* of Colombia **(20)**. Overall, the virulence of these strains was less in Panamanian than in Colombian owl monkeys, as indicated by lower mortality rates of Panamanian monkeys during the first 30 days of patency. Maximum parasitemias of the Vietnam Smith and Uganda Palo Alto strains were, however, significantly higher during the first 15 days of patency in Panamanian than in Colombian owl monkeys. These quantitative differences in infection parameters between Panamanian and Colombian owl monkeys have not invalidated the use of the former for evaluation of new antimalarial drugs.

Numerous candidate antimalarial drugs of diverse chemical classes have been evaluated against trophozoite-induced infections of one or more *P. falciparum* strains during the course of these contracts. In seeking alternatives to primaquine, two 8-aminoquinolines proved to be active against the blood stages of *P. falciparum* **(2, 13)**. Desferrioxamine, an iron-specific- chelating agent, was shown to suppress parasitemias of the

virulent Uganda Palo Alto strain of *P. falciparum* **(18)**. The *in vitro* activity of two halogenated histidine analogs was not confirmed by evaluation against *P. falciparum* infections in owl monkeys **(17)**.

Chloroquine-resistance of *P. falciparum* represents the greatest challenge in developing effective antimalarial drugs. Reversal of chloroquine-resistance in *P. falciparum, in vitro*, was achieved by the co-administration of verapamil (a calcium channel blocker) plus chloroquine **(12)**. Other in vitro studies have shown that there is a significantly greater efflux of chloroquine from erythrocytes containing falciparum parasites resistant to chloroquine than from red cells parasitized by chloroquine-sensitive falciparum malaria **(9)**. Calcium channel blockers appear to prevent this active efflux of chloroquine, thus allowing the drug to accumulate to parasiticidal levels.

Based upon the success of *in vitro* reversal of chloroquine-resistance, trials were initiated to determine if resistance could be reversed in *Aotus* infected with the chloroquine-resistant Vietnam Smith strain of *P. falciparum*. Six calcium channel blockers, or similarly acting drugs, were co-administered with chloroquine in diverse regimens. The desideratum of chloroquine-resistance reversal was administration of a single course of treatment, with parasite clearance and infection cure. Suppression of parasitemia was obtained during an initial course of treatment, but parasite clearance and cure occurred in some instances only after re-treatment. Such infection parameters were similar to those in monkeys with self-limited infections and cure could be attributed to acquired immunity.

Limited trials with desipramine, Norpramin , a tricyclic psychotropic drug, demonstrated the feasibility of reversing chloroquine-resistance in vivo **(1)**. Parasite clearance was obtained, but the infection was not cured.

Subsequently, in vivo reversal of chloroquine resistance was obtained with combinations of chloroquine plus chlorpromazine or prochlorperazine. Such reversal was exhibited by rapid suppression and clearance of parasitemia, resulting in infection cure without retreatment **(10)**.

Evaluation of two oil-soluble derivatives of artemisinin, artemether and arteether, demonstrates that both possess similar activity to cure infections of a multi-drug resistant *P. falciparum* strain in *Aotus* **(23)**.

Some strains of *P. vivax* from the Melanesian and Indonesian archipelago have demonstrated resistance to treatment with chloroquine **(14, 19)**. Unlike chloroquine-resistant falciparum malaria, there exists no easy alternative to chloroquine-resistant strains of vivax malaria. Using WR 238605 alone or in combination with chloroquine in Panamanian *Aotus* monkeys it was demonstrated that WR238605 is an alternative treatment for chloroquine-resistant vivax malaria **(16)**. The compound WR 238605 is a primaquine analog developed by the US Army as a better tolerated, more effective replacement for primaquine.

Both the purpose and methods of approach of the present work remains essentially unchanged since 1976, viz to ascertain the antimalarial activity of drugs against *P. falciparum* and *P. vivax* in *Aotus*. The method of approach may vary on an ad hoc basis, such as administering a combination of drugs.

The long term goal of the second part of this project is to develop fully protective plasmid DNA vaccines that induce protective immune responses against the sporozoite, liver and erythrocytic stages of *P. falciparum*. If successful, it will establish, for the first time, that plasmid DNA vaccines can protect non-human primates, a critical step forward for the use of plasmid DNA vaccines in humans.

Vaccines are aimed at inducing immune responses that disrupt the complex cycle of the parasite at one or more points: anti-sporozoite antibodies that prevent invasion of hepatocytes; cytotoxic T lymphocytes, cytokines, and antibodies that eliminate infected hepatocytes; antimerozoite antibodies that prevent invasion of erythrocytes; antibodies that neutralize parasite exoantigens known to induce harmful cytokine responses; antibodies that attack infected erythrocytes; cytokines that kill parasites within erythrocytes; and, anti-sexual stage antibodies that prevent the development of sporozoites in the mosquito.

Previous trials of malaria blood stage vaccines have shown that the Panamanian *Aotus**P. falciparum* model to be suitable for this purpose. **(6-8)**.

Immunogenicity studies of a plasmid DNA vaccines encoding the circumsporozite *P. yoelli* rodent malaria gene (PyCSP) in Panamanian *Aotus* monkeys demonstrated that the intradermal route of inoculation (ID) induces a higher level of antibodies than the intramuscular route (IM). Antibody levels induced in this manner reached a peak at week 9 and titers declined to 50% their peak value by week 14. When boosted at week 46 antibody levels increase 4 fold by week 49. This was comparable to antibodies generated with a Multiple Antigen synthetic peptide vaccine (MAP) delivered with an adjuvant **(4)**

We have used this immunization scheduled to test single or multi-gene DNA plasmid vaccines in Aotus monkeys. Additionally we have tested the ability of recombinant cytokines to enhance the immunogenicity and protective efficacy of the DNA vaccines. Preliminary studies (previously described in the 1996 Annual Report) using a small group of *Aotus l. lemurinus* (n=3) demonstrated partial, but incomplete, protection with a DNA vaccines for either AMA-1 or EBA-175 alone. These studies indicated that animals which received the vaccine candidates, had a short, but apparent significant delay in the onset of parasitemia {approximately 33% (1 of 3) self-cured, whereas none of the control animals did}. However, since the number of animals per group in each of these pilot studies were small, it was not possible to determine the absolute efficacy of these candidate

vaccines, but these experiments suggested to the investigators that further studies were warranted. MSP-1, when used as a protein/peptide vaccine formulation, provided protection from a *P. faciparum* infection in Aotus monkeys and we have demonstrated that, in mice and in Rhesus monkeys, the cytokine GM-CSF augmented both immunogenicity of a malaria DNA vaccine (personal communication. W. Weiss). We have now completed a pilot experiment to determine if Aotus Granulocyte-Macrofage-Colony Stimulating Factor (aGM-CSF) can augment immunogenicity and protective efficacy of a multi-gene erythrocytic vaccine.

The effect of prior *P. falciparum* infection on the immunogenicity of a DNA vaccine is unknown. Hence, we tested the immunogenicity and protective efficacy of the multi-gene erythrocytic vaccine in monkeys that have had one prior *P. falciparum* infection.

We are also testing a multicomponent *P. vivax* DNA vaccine which includes two pre-erythrocytic stage antigens, PvCSP and PvSSP2, which are the target of protective immune responses in *P. falciparum* and *P. yoelii*, and three erythrocytic stage antigens, PvMSP1(p42), PvAMA1, and PvDBP(regions II-IV). The rationale for the vaccine is that immune responses to the two pre-erythrocytic stage vaccines will neutralize sporozoites or, failing that, eliminate infected hepatocytes. If any hepatic schizonts do develop, the resulting blood stage infection will be controlled by responses to the three erythrocytic stage antigens. The N-terminal fragment of MSP1, p42, is a target of protective immune responses in P. falciparum and *P. yoelii*, and is believed to have a role in invasion of erythocytes. AMA1 is a protein located in the apical organelle complex which is released during the invasion process, and may have a role in invasion. The regions II-IV of the PvDBP are the regions involved in binding of merozoites to the Duffy receptor on the surface of Duffy+ erythrocytes required for invasion *by P. vivax*. The five *P. vivax* DNA vaccines have been tested for immunogenicity in outbred, CD-1 mice. The immune sera from these mice were tested in IFA against *P. vivax* infected erythrocytes which we prepared from the blood of Aotus infected with the Sal I strain of *P. vivax*, and against Sal I sporozoites prepared by Dr. W. Collins, Centers for Disease Control and Prevention in Atlanta. All five DNA vaccines were found to induce specific antibodies with titers up to 1:20,000 after two doses.

In Aotus monkeys it is possible to test the immunogenicity of all five components of the multicomponent vaccines. The protective efficacy of the multicomponent DNA vaccine against blood stage parasites can be readily tested in *A. l. lemurinus* monkeys which are susceptible to the Sal I strain of *P. vivax* as demonstrated in this report. It is important to include the pre-erythrocytic stage antigens in such a challenge for two reasons. First, they serve as an additional negative control when compared to the erythrocytic stage antigens. Second, there has been some concern that inclusion of

multiple antigens in a multi-component vaccine will diminish the immune responses to each of the individual components. Inclusion of the two pre-erythrocytic stage antigens will allow this point to be addressed. Unfortunately, there is no adequate sporozoite challenge model for *P. vivax* in Aotus. Therefore the protective efficacy of the pre-erythrocytic stage components will need to be tested separately at a later date.

The purpose of this report is to: 1) Present data on the evaluation of potential antimalarial activity of drugs in the pre-clinical model of *Aotus l. lemurinus* (Panamanian night monkey) experimentally infected with *P. falciparum* or *P. vivax*, and 2) data on plasmid DNA malaria vaccine experiments. These studies were supported by the U.S. Army and the U.S. Navy Malaria Programs.

BODY

I. Experimental Methods

The first aim of this project is to evaluate the potential antimalarial activity of drugs, or combination thereof, in the preclinical model of *Aotus* experimentally infected with *P. falciparum* (or *P. vivax*). Specifically, the vertebrate host is *A. l. lemurinus*, the Panamanian night monkey. These animals are either feral, laboratory adapted or laboratory born. No naturally acquired, human plasmodium infection has been reported in *Aotus*. The Vietnam Smith/RE strain of *P. falciparum* was adapted to *Aotus* of Colombian origin in 1971 **(21)** and in Panamanian *Aotus* in 1976. **(20)**. The course of untreated infections, essential for comparison with treated infections, has been documented in Panamanian *Aotus* **(20)**. This plasmodium strain is resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine **(22)**.

To initiate an experiment, infected blood (with 2.5% sodium citrate as the anticoagulant) from an untreated *Aotus* was diluted appropriately in chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites. This amount was inoculated into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm. **(3)**

Blood films from untreated *Aotus*, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If recrudescence occurred, blood films were obtained again on a daily basis.

Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solutions of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8° C for the treatment period. If

a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was by gastric intubation with a 14 French catheter. The total volume of fluid administered, drug solution or suspension, and rinse was 14 ml.

Response to treatment was categorized as clearance and cure, clearance and recrudescence, or suppression without clearance. The day of clearance was defined as the first of three consecutive days in which the thick blood films were parasite negative. The day of recrudescence was the first of three consecutive days of positive thick blood films after a period of clearance. Suppression was defined as a transient decrease in the parasite count post-treatment without clearance.

The second objective of this project is to evaluate plasmid DNA vaccines against the blood and sporozoite stages of *P. falciparum* and against the blood stages of *P. vivax* in the Panamanian *Aotus* model. To this end we have evaluated single and multigene DNA vaccines of *both P. falciparum* and *P. vivax* with or without the addition of cytokines. The results of these experiments are detailed in results.

II. Results

A. Adaptation of a *P. falciparum* strain 1088 to Panamanian *A. l. lemurinus* monkeys.

In an attempt to adapt a *P. falciparum* 1088 strain to Panamanian *A. l. lemurinus* one malaria naive splenectomized monkey was inoculated intraperitoneally (IP) with frozen blood sent from WRAIR on 24 June 1997. This animal remained negative for more than 100 days post-inoculation (PI).

B. Establishment of *P. vivax* Salvador I (PvSal I) strain in splenectomized and intact Aotus monkeys and extraction of *P. vivax* RNA for DNA cloning.

On 16 May 1997, one *P. falciparum* cured splenectomized Aotus was inoculated IV with 1.25 ml of frozen and washed Pv Sal 1 Aotus infected red cells. When parasitemia was near its peak 15 days after inoculation, four additional splenectomized monkeys were infected with 5 X 10^6 parasitized erythrocytes, IFA slides and cryopreserved blood were prepared at this time. Recipient monkeys were bled 10 days PI, 5 mls each and their blood transported the same day to NMRI in Rockville, MA, where RNA extraction was performed 14-16 hours thereafter.

On January 9, 1998 one intact *P. falciparum* cured Aotus was inoculated IV and IP with a frozen stock of *P. vivax* Sal 1 strain passaged in splenectomized animals. When the animal reached 4.3 x 10^6 parasites x ml on day 12 the parasite was further passaged into an intact *P. falciparum* cured Aotus, this time the animal peak on day 8 PI with 4.3 x 10^6 parasites x ml. Further passages were done in four additional intact monkeys until the parasitemia peak and stabilized at around 20,000 parasites x *ul* on day 12 PI. Only one of six animals self-cured and the others, either had recrudesences or low grade parasitemias <10 parasites x *ul* until the moment of this report.

C. Toxicity of an oral route of administration of WR255663AK (JN8331), Artelinic acid in Aotus.

Artelinic acid an Artemisinin derivative is known to possess in vitro and in vivo antimalarial activity against strains of *Plasmodium falciparum* and *Plasmodium berghei*. In order to test Artelinic acid toxicity by the oral route in an Aotus monkey-model, on 12 August 1997, one Aotus (weighing 983 grms) cured of malaria infection was administered 20 mg/kg of WR255663AK (JN83331) Artelinic Acid orally in 5% sodium carbonate pH 8.4, twice daily for three consecutive days. During treatment the animal

was monitored for weight loss, depression, anorexia, vomiting or neurological signs. Apart from a transient loss of 13% body weight, which was gradually recovered over a month period, no other side effects were observed during treatment and follow up.

D. Efficacy of an oral route of administration of WR255663AK (JN8331), Artelinic acid against a *P. falciparum* FVO strain infection in Aotus.

In a toxicity study shown above, an oral dose of 20 mg/kg of WR255663AK (JN8331) Artelinic acid administered orally, twice a day for three days proved to be safe when tested in Aotus. On 5 September 1997, one malaria naive Aotus (weighing 823 grams) which had been infected with 1 ml of frozen *P. falciparum* FVO strain IP was treated orally with 20 mg/kg of WR255663AK (JN8331) Artelinic acid in 5% sodium carbonate pH 8.4 for three consecutive days, beginning on the day when parasitemia reached 5,000 parasites per cmm. As shown in Table 1 and 2 parasitemia cleared three days after initiation of treatment, but a recrudecence occurred 31 days PI with a peak parasitemia of 289,000 parasites x cmm on day 38 PI when retreatment was initiated, this time at 40 mg/kg of WR255663AK (JN8331) Artelinic acid orally, twice daily for three consecutive days. Parasite clearance and cured occurred on day 42 PI, four days after initiation of treatment. The animal remained negative up to day 100 PI when the experiment was terminated.

E. Immunogenicity and Efficacy of a *P. falciparum* EBA-175, AMA-1 and MSP-1 DNA Vaccine alone or in combination in Aotus Monkeys.

Forty malaria naive Aotus were divided into five groups of eight monkeys each and immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines alone or in combination three times at monthly intervals and then boosted at six months, in order to determine its immunogenicity and efficacy.

Results of the first challenge for groups 1, 2 and 3, carried out on August 12, 1997 with 1 x 10^5 parasites of *P. falciparum* FVO, were considered invalid when groups 4 and 5 plus a naive control failed to developed infection 56 days after inoculation. To overcome the unexpected loss of infection in Groups 4 and 5, which might have been due to a die off of the parasite, as it is presumed from an observed delay in patency in groups 1, 2 and 3 as shown in Table 3. It was collectively decided to modify the challenge procedure in the following way: Media for inoculation was changed from chilled saline to RPMI and all procedures were carried out at room temperature. Groups 4 and 5 were then re-vaccinated

on October 8, fifty six days after inoculation and rechallenged on 28 October, seventy seven days after the first challenge with 1 x 10^5 parasites of the FVO strain. This time as shown in table 3a, all animals in Groups 4 and 5 became parasitemic with no detectable differences in pre-patent period, day to peak parasitemia or day of initiation of treatment with mefloquine. Therefore the vaccine candidates did not have any demonstrable effect on the course of parasitemia in these animals.

F. Immunogenicity and Efficacy of a *P. falciparum* EBA-175, AMA-1, MSP-1 DNA Vaccine as a combination with or without Aotus Granulocyte-Macrofage-Colony-Stimulating Factor (aGM-CSF) in Aotus Monkeys.

Twelve malaria naive Aotus were divided into four groups of 3 monkeys each and immunized intradermally with a combination erythrocytic stage malaria plasmid DNA vaccine consisting of EBA-175, MSP-1 and AMA-1 with or without co-delivery of an expression plasmid encoding an Aotus aGM-CSF, three times at monthly intervals and then boosted at six months, in order to test its immunogenicity and efficacy. Challenged with 1 x 10^5 parasites of a *P. falciparum* FVO strain was carried out on January 19, 1998. As Shown in Table 4 all animals were patent between days 6 and 7 PI. A naive control was treated with mefloquine on day 12 PI when reached 400,000 parasites x *ul*. On day 13 PI one animal from group 1, two animals from group 2, three from group 3 and two from group 4 were treated as well. Additionally, two animals from group one were treated on day 14 PI. Although, the remaining two animals, one from 4 and the other one from group 2, did not reach the 400,000 parasites x *ul* limit, both had to be treated on days 17 and 18 PI respectively, due to low hematocrit readings. Therefore, it could be concluded from this experiment that the candidate vaccines did not protect the monkeys against challenge.

G. Immunogenicity and efficacy of a *P. falciparum* EBA-175, AMA-1 and MSP-1 DNA vaccine as a combination in PfFVO single-cured Aotus monkeys.

Twelve single cured PfFVO Aotus monkeys were divided into two groups of six monkeys each and immunized intradermally three times at monthly intervals and then boosted at six months, in order to compare the immunogenicity and protective efficacy of a combination erythrocytic stage plasmid DNA malaria vaccine consisting of EBA-175, MSP-1 and AMA-1.

All animals were challenged with 1 x 10^5 parasites of a *P. falciparum* FVO strain on January 19, 1998. As shown on table 5 one animal from group 1 an another one from group 2, were first patent on day 8 PI, and both had to be treated due to low Htos on days 23-27 PI respectively. In addition, 3/6 monkeys from group 1 became patent between days 15-17 PI, of these, one was only transiently parasitemic between days 14-17 PI, with

<10 parasites x *ul* of blood, and then recrudesce on days 30-36 remaining negative until day 56. Another one was also transiently parasitemic between days 14-17PI and then self-cured. The other one, had to be treated with mefloquine on day 24 PI due to a low Hto. The remaining two animals of this group remained negative for more than 56 days PI. In group two, 5/6 animals became positive between days 8-16, of these, one animal remained negative for more than 56 days PI and another one cleared its parasitemia on day 17 PI, but recrudesce between days 28-35 PI, self-curing on day 36 PI. The other four had to be treated with mefloquine between days 22-27 PI. One of these animals died of malaria related complications, even though its parasitemia was < 10 parasites x *ul* during the course of the experiment. Therefore, it is concluded that complete or partial protection was achieved in 4/6 (67%) monkeys from group 1 that received the triple combination vaccine, compared to only 2/6 (33%) in the control group.

H. Immunogenicity and Efficacy of *P. vivax* DNA Vaccines based on PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP(regionsII-IV) alone or in combination in Aotus Monkeys.

Thirty six animals were divided in four groups of three and three groups of eight animals respectively and immunized intradermally, in order to evaluate the immunogenicity and efficacy alone or in combination of five components of a multi-component DNA vaccine against *P. vivax*, PvCSP, PvSSP2, PvMSP1(p42), PvMA1, PvDBP(regions II-IV). The first four groups were immunized with a PvCSP (Group 1), PvSSP2 (Group 2), MSP-1(p42) (Group 3), AMA-1 (Group 4). The primary purpose of these three groups was to test immunogenicity of these four individual components. The final three groups included 8 monkeys each and were immunized with PvDBP(regions II-IV) (Group 5), a mixture of the five individual plasmids (Group 6), and a negative control plasmid (Group7).

At the moment of this report no antibody responses have been observed in all animals after three immunizations.

I. Induction of immunity by repeated challenge with the FVO strain of *P. falciparum.*

Of the various *P. falciparum* strains adapted to non-human primates, the FVO (Vietnam-Oak Knoll) strain would be useful for vaccine studies as only 25-30% of infected Panamanian *Aotus* self-cure (20). The remainder of the infected animals require curative drug treatment or death will ensue. When evaluating a vaccine, the higher the proportion of self-cure, the greater the number of animals needed in each experimental group to assure that the animals are protected by the vaccine and not self-curing.

To compare the efficacy of an "artificial" vaccine with protection afforded by acquired immunity, an experiment was initiated to induce immunity by repeated trophozoite challenge. Initial results were given in the previous report. Briefly, malaria naive Panamanian *Aotus* were inoculated with 10,000 parasites of the FVO strain, the parasitemia monitored daily by blood film examination, and the infection cured with mefloquine (40.0 mg/kg, oral) when parasitemia approximated 400,000 per cmm. About 4 to 6 weeks after infection cure, the animals will be rechallenged with parasites from a donor monkey whose infection was initiated by cryopreserved parasites. Donor animals, cured of infection, were recycled into the challenge group. Challenges will be repeated until the monkeys demonstrate complete immunity.

The current results summarized in Table 6 indicate that sterile immunity has been induced in thirteen Aotus following 4-6 rechallenges, being the last one on 19 January, 1998. Two of these animals died of intercurrent infection during fiscal year 96-97. Following this homologous rechallenge, a heterologous challenge is planned with a plasmodium strain yet to be determined. At the moment of this report only 1/8 animals have become parasitemic on day 7 PI with a peak on day 10 of 620 parasites x ul and self cured on day 16 PI.

CONCLUSIONS

A frozen *Plasmodium falciparum* strain 1088 did not adapt when inoculated in Panamanian *A. l. lemurinus* monkeys by the IP route.

A Salvador I (PvSal I) strain of *P. vivax* was successfully adapted in splenectomized and intact *A. l. lemurinus* monkeys after serial in *vivo passage*.

Artelinic acid WR255663AK (JN8331) when given to Aotus monkeys by the oral route at 20 mg/kg twice daily for three consecutive days, appeared to be safe, and cleared a *P. falciparum* FVO infection three days after initiation of treatment. Re-treatment at 40 mg/kg cured a recrudecence that occurred 31 days PI.

Neither Aotus immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines alone or in combination, nor Aotus immunized intradermally with AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines in combination with or without Aotus Granulocyte-Macrofage-Colony-Stimulating Factor (aGM-CSF) were protected when challenged with an FVO strain of *P. falciparum.*

AMA-1, EBA-175 and MSP-1 plasmid DNA vaccines when given intradermally as a combination to *P. falciparum* FVO cured Aotus protected 4/6 (67%) monkeys against an homologus re-challenge, in contrast to 2/6 (33%) in the control group.

Total absence of antibody responses were observed in single cured *P. falciparum* Aotus monkeys immunized intradermally after three immunizations with *P. vivax* DNA vaccines based on PvCSP, PvSSP2, PvMSP-1p42, PvAMA1, and PvDBP(regionsII-IV) alone or in combination.

Homologus re-challenge with Vietnam-Oak Knoll parasites has, to date, resulted in thirteen Aotus with sterile immunity after 4-6 inoculations. These animals, as well as others without such immunity will be re-challenge both with a heterologous strain. Data will be compared with a hopefully effective DNA vaccine.

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TABLE 1

DETAILED ACTIVITY OF ARTELINIC ACID WR255663AK (JN8331) AGAINST INFECTIONS OF THE FVO STRAIN OF *PLASMODIUM FALCIPARUM* IN AOTUS.

				PARASITEMIA PER cmm X 10^3									
				DAY OF TREATMENT			DAYS POST TREATMENT						
AOTUS NO.	DAY PAT,	MG/KG DOSE	DAY PRE RX	1	2	3	1	2	3	4	5	6	7	DAYS Neg.
12893	3	20	16.2	5.6	1.5	<10	0	0	0	0	0	0	0	20
12893*	31	40	227.9	289.4	123.2	33.8	<10	0	0	0	0	0	0	59

*=Retreatment

TABLE 2

SUMMARY OF ACTIVITY OF ARTELINIC ACID WR255663AK (JN83331) AGAINST INFECTIONS OF THE FVO STRAIN OF *PLASMODIUM FALCIPARUM* IN AOTUS

Monkey No.	Daily Dose x 7 Mg/Kg	Response of Parasitemia to RX			Days from initial Rx to parasite Clearance	Days from final Rx to Recrudes-cence	Notes No. of days negative
		None	Suppresed	Cleared			
12893	20			X	3	21	21
12893*	40			X	4	0	59

*=Retreatment

TABLE 3

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 ALONE AND CHALLENGED WITH A P. FALCIPARUM FVO STRAIN

Parasites x cmm

PI/DAY

Monkey No.	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12835	1	0	0	0	0	0	<10	<10	<10	49280	58520	221760	308000	314160	401090*		
12836	1	0	0	0	0	0	<10	<10	<10	18440	21560	105610	213000	272400	543920*		
12837	1	0	0	0	0	0	0	0	0	0	<10	<10	<10	3110	60160	97940	320400
12838	1	0	0	0	0	0	0	0	0	0	0	0	0	1060	980	36960	348110
12840	1	0	0	0	0	0	<10	<10	<10	<10	10780	140940	235500	326480	309940	408000	351120
12852	1	0	0	0	0	0	<10	<10	<10	15400	19890	117040	380000	430480	566720*		DIED
12841	1	0	0	0	0	0	0	0	<10	<10	7710	89970	106500	217920	469800*		
12844	1	0	0	0	0	0	0	0	<10	<10	3010	30010	99000	84160	289520	240960	394260*

Monkey No.	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12877	2	0	0	0	0	0	0	0	0	<10	<10	2970	480	86390	47790	537440*	
12845	2	0	0	0	0	0	0	0	<10	<10	740	7690	2380	132420	314160	304960	763440*
12846	2	0	0	0	0	0	0	0	<10	<10	970	147860	109500	301840	283340	283940	409580*
12847	2	0	0	0	0	0	0	0	<10	<10	1010	66220	90000	331200	375760	334080	689920*
12848	2	0	0	0	0	0	0	0	<10	<10	490	47090	102000	194800	264880	341760	389520*
12860	2	0	0	0	0	0	0	0	<10	0	<10	<10	<10	<10	<10	21560	70010
12849	2	0	0	0	0	0	0	0	<10	<10	<10	4620	1180	151680	283360	482110*	
12851	2	0	0	0	0	0	0	0	0	0	0	0	0	<10	<10	1620	28820

Monkey No.	Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
12850	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	1590	7700	95440
12855	3	0	0	0	0	0	0	0	0	0	0	0	0	<10	<10	13860	13860
12856	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	18460	50820	271040
12857	3	0	0	0	0	0	0	0	0	0	0	0	0	<10	1110	980	13010
12858	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12859	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	40610	47750	320370
12861	3	0	0	0	0	0	0	0	0	0	0	0	<10	<10	19850	33880	368010
12862	3	0	0	0	0	0	0	0	0	0	0	0	0	0	<10	<10	<10

* = Treatment with mefloquine

Group 1 = AMA-1

Group 2 = EBA-175

Group 3 = MSP-1

CONT... TABLE 3

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 ALONE AND CHALLENGED WITH A P. FALCIPARUM FVO STRAIN

Parasites x ccmm

PI/DAY

Monkey Number	Group	17	18	19	20	21	22	23	24	25	26
12835	1										
12836	1										
12837	1	431200*									
12838	1	581040*									
12840	1	501160*									
12852	1										
12841	1										
12844	1										

Monkey Number	Group	17	18	19	20	21	22	23	24	25	26
12877	2										
12845	2										
12846	2										
12847	2										
12848	2										
12860	2	338800	466720*								
12849	2										
12851	2	15460	361280	201000	528000*						

Monkey Number	Group	17	18	19	20	21	22	23	24	25	26
12850	3	110990	375760	288000	576000*						
12855	3	196340	369600	214500	326000	291500	576000*				
12856	3	437360*									
12857	3	78540	96400	125000	64500	73500	73500	127500	68750*		
12858	3	0	0	0	0	0	0	0	0	0	0
12859	3	399600*									
12861	3	560070*									
12862	3	920	123200	576000*							

* = Treatment with mefloquine

Group 1 = AMA-1

Group 2 = EBA-175

Group 3 = MSP-1

TABLE 3a

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION AND CHALLENGED WITH A P. FALCIPARUM FVO STRAIN

Parasites x cmm
Day/PI

Monkey No.		6	7	8	9	10	11	12	13	14	15	16
12863	4	<10	<10	190	16820	66000	364500	812050*				
12865	4	0	<10	80	17760	43500	341250	1094900*				
12866	4	0	<10	<10	12900	38700	152250	466370*				
12869	4	0	<10	90	1980	19500	88500	372000	492000*			
12870	4	0	<10	240	8320	40500	141000	512560*				
12872	4	0	<10	90	2440	16500	67800	202500	246250	388010	197120	458560*
12873	4	0	<10	70	4370	18300	141750	594510*				
12875	4	0	<10	50	2780	5110	49920	355500	693750*			

Monkey No.		6	7	8	9	10	11	12	13	14	15	16
12879	5	0	<10	160	11360	81000	179250	477160*				
12822	5	0	0	<10	7650	16900	130500	339720	813400*			
12823	5	0	<10	<10	9550	28770	136500	586120*				
12829	5	0	0	<10	9350	47850	124450	421500*				
12830	5	0	0	<10	4820	20100	81750	354750	480750*			
12832	5	0	0	<10	1420	2520	62250	242250	310500	402000*		
12878	5	0	<10	180	3750	9080	76540	300750	1299280			

Monkey No.		6	7	8	9	10	11	12	13	14	15	16
12880	Control	<10	<10	330	29880	109500	407250*					
12896	Control	<10	<10	110	38000	99000	293250	655600*				
12897	Control	<10	<10	<10	1940	5830	31800	315750	389250	378640	369660	442080*
12898	Control	0	<10	100	3180	73500	106500	560250*				

*=Treatment with mefloquine
Group 4= Combination vaccine
Group 5= Plasmid control
Group Control= Malaria Naive

TABLE 4

DETAILED PARASITEMIA OF AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION WITH OR WITHOUT aGM-CSF AND CHALLENGED WITH A P. FALCIPARUM FVO STRAIN

Parasites x cmm
DAY/PI

Group		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Monkey Number																			
12876	1	0	0	0	0	0	0	<10	>10	27940	8790	160160	220960	308000	382,240	366480*			
12882	1	0	0	0	0	0	0	<10	<10	6700	3080	157010	209420	332680	304,920	510000*			
12883	1	0	0	0	0	0	0	<10	<10	6158	21560	295680	226400	542080*					
12884	2	0	0	0	0	0	0	<10	>10	10780	7720	252160	277200	590910*					
12885	2	0	0	0	0	0	0	<10	>10	18480	9240	285610	303840	569930*					
12886	2	0	0	0	0	0	0	<10	>10	>10	1060	18690	23100	30800	47,250	25710	9240	30800	48750*
12887	3	0	0	0	0	0	0	<10	<10	>10	20020	135520	166320	539110*					
12888	3	0	0	0	0	0	0	<10	<10	>10	980	135520	158020	401220*					
12890	3	0	0	0	0	0	0	<10	<10	>10	4620	115760	120190	576720*					
12889	4	0	0	0	0	0	0	<10	<10	>10	13960	224800	78540	331010	243000	384010	258720	314160*	
12891	4	0	0	0	0	0	<10	<10	>10	27720	13860	246400	120190	517440					
12892	4	0	0	0	0	0	0	<10	<10	>10	710	151720	136280	325520	408000*				
12901	CONTROL	0	0	0	0	0	<10	<10	>10	9240	6060	207120	470400*						

*= Treatment with mefloquine
Group 1= Triple combination without aGM-CSF
Group 2= Triple combination with aGM-CSF
Group 3= Plasmid control with aGM-CSF
Group 4= Plasmid control without aGM-CSF

TABLE 5

DETAILED PARASITEMIA OF P. FALCIPARUM FVO CURED AOTUS VACCINATED WITH A PLASMID DNA VACCINE EBA-175, AMA-1, MSP-1 AS A COMBINATION AND RE-CHALLENGED WITH AN HOMOLOGUS STRAIN

Parasites x cmm

Monkey No.	Group	Day/PI 8	9	10	11	12	13	14	15	16	17	18
12771	1	0	0	0	0	0	0	0	<10	<10	<10	0
12772	1	<10	<10	<10	<10	<10	<10	>10	10780	283360	73920	127820
12773	1	0	0	0	0	0	0	0	0	0	0	0
12774	1	0	0	0	0	0	0	>10	3080	21560	27720	39910
12775	1	0	0	0	0	0	0	>10	860	1010	<10	0
12778	1	0	0	0	0	0	0	0	0	0	0	0
12779	2	<10	<10	<10	0	0	0	0	<10	<10	<10	<10
12781	2	0	0	0	0	0	0	>10	1510	1260	980	5970
12782	2	0	0	0	0	0	0	0	<10	<10	<10	0
12783	2	0	0	0	0	0	0	0	0	0	0	0
12784	2	0	0	0	0	0	0	0	0	<10	<10	0
12785	2	0	0	0	0	0	0	0	<10	<10	<10	<10

Monkey No.	Group	Day/PI 19	20	21	22	23	24	25	26	27	28	29	30-35
12771	1	0	0	0	0	0	0	0	0	0	0	0	<10
12772	1	124740	169400	82550	1893	308*							
12773	1	0	0	0	0	0	0	0	0	0	0	0	0
12774	1	27810	38990	20450	318	124	790*						
12775	1	0	0	0	0	0	0	0	0	0	0	0	0
12778	1	0	0	0	0	0	0	0	0	0	0	0	0
12779	2	<10	>10	1580	1497	1609	28750	59060	48810	184800*			
12781	2	7920	1908	5580	116	690*							
12782	2	0	0	0	0	0	0	0	0	0	<10	<10	<10
12783	2	0	0	0	0	0	0	0	0	0	0	0	0
12784	2	0	0	<10	>10*	<10	0	DIED/malaria					
12785	2	<10	<10	10	390	<10	0	0*					

Group 1= AMA-1, EBA-175, MSP-1
Group 2= Plasmid control
*=Treated with mefloquine

TABLE 6

CHALLENGE WITH THE FVO STRAIN OF *PLASMODIUM FALCIPARUM*

MONK NO.	NO. OF CHALLENGES	NOTES
12730	6	Sterile immunity
12735	6	Sterile immunity
12739	6	Sterile immunity
12749	6	Sterile immunity
12756	6	Sterile immunity
12757	6	Sterile immunity
12759	6	Sterile immunity
12763	6	Sterile immunity
12765	6	Sterile immunity
12762	5	Sterile immunity
12727	6	Sterile imm./died pneumonia
12748	4	Sterile imm./died interc. infect.
12752	4	Not immune/Died/49 days/PI
12794	4	Sterile immunity
12821	4	Not immune
12764	3	Died Malaria/25 days/PI
12169	2	Died day 32 days/PI, malaria
12687	2	Rx,died day 46 days/PI, inter-current infection
12738	2	Died day 19/PI, malaria
12740	2	Rx,died 51 days/PI inter-current infection
12731	1	Died of Malaria 17 days/PI
12726	1	Died of Malaria 18 days/PI
12761	1	Died of intercurrent infection 46 days/PI
12768	1	Died lung aspiration17 days/PI
12786	2	Died/Malaria 23 days/PI